

**TITLE:** SINGLE GENE CASSETTE WITHIN CLASS 1 INTEGRON FROM MULTIDRUG-RESISTANT *Escherichia coli* CARRIED BY FLIES

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**ABSTRACT:**

Integrations are genetic elements that act in gene cassettes integration by site-specific recombination. Due to the aggregation of antimicrobial resistance gene cassettes, and the association with mobile elements, resistances integrons are have been related to the antimicrobial spread in the environment. In this regard, we aimed to characterize the class 1 integron gene cassettes present in *Escherichia coli* multidrug resistant ( $\beta$ -lactam, quinolone, chloramphenicol/aminoglycoside, sulphonamide + folate pathway inhibitor, and tetracycline), isolated from the external surface of flies collected in a milking environment. Strains were analyzed for antimicrobial susceptibility by disk diffusion test and the presence of class 1 integron genes by PCR (*int1* and cassette genes region 5'CS-3'CS). From 57 flies were isolated 135 *E. coli*, with eight strains (5.9%) showing class 1 integron and multidrug resistance to five of the six antimicrobial classes tested. The sequencing analysis showed the same gene cassette in the eight strains with only one gene within to class 1 integron. The gene cassette found was *dfrA7* gene that confers trimethoprim resistance. In order to confirm the integron structure, linkage PCRs were performed (combining the primers *int1*, *dfrA7*, and *qacEΔ1*), which resulted in the *int1-dfrA7-attC-qacEΔ1* structure. The *dfrA7* gene exhibited alternative start codon "GTG", and its recombination site 143bp. The integron-positive *E. coli* were isolated from two flies' species (*M. domestica* = 5 strains; *Fannia* sp. = 3 strains) which, despite being associated with cattle feces, circulate in different rural and urban environments. The arrangement containing only *dfrA7* within integron is not common, so the detection of this arrangement in *E. coli* from different flies may indicate the regional spread of this gene cassette. However, further studies should be conducted to substantiate this hypothesis.

**Keywords:** *dfrA7* gene, dairy farm, antimicrobial resistance

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